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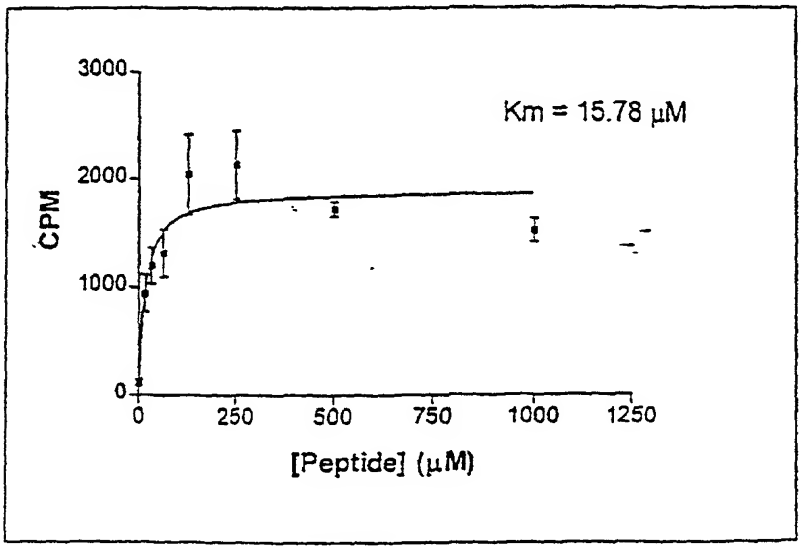
(54) Title: A METHOD FOR MAPPING THE ACTIVE SITES BOUND BY ENZYMES THAT COVALENTLY MODIFY SUBSTRATE MOLECULES

(57) Abstract

This invention provides for the active site mapping of enzymes which catalyse covalent modification including, but not limited to phosphorylation, acylation, dephosphorylation in which a fixed residue (known as the catalytic residue) such as a tyrosine, serine, threonine, histidine, aspartic acid residue or any other residue containing an appropriate side chain is modified. Mapping of protein kinases is exemplified. The method of the invention has an additional level of complexity over and above that of the self–deconvoluting libraries described in WO97/42216. This involves making a library of smaller libraries (referred to as subsets) where a fixed residue is moved stepwise through the sequence of amino acids or other groups (such as peptidomimetics). Using 5 subsets of libraries of peptides of 5 amino acids allows the mapping of a sequence of 9 amino acids. In general one could carry out the invention using n subsets of n–mer peptides so as to provide mapping data for the residues from –(n–1) to +(n–1) either side of the active site. Thus in general the length of the mapped sequence would be (2n)–1. In this invention there is no need to separate modified from unmodified sequences because of the self deconvoluting nature of the library. The assay screen produces a series of hits, the patterns of which reveal the unique sequences in each well. This enables a pattern of substrate preferences to be determined for any enzyme. The unique sequences obtained using this invention can be used to provide substrates for high throughput assays and provide detailed information about the active site to aid rational drug design. This invention can also be used as an inhibitor library to screen against known modifying enzymes where a known substrate exists and can be set up in an assay format.



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(54) Title: A METHOD FOR MAPPING THE ACTIVE SITES BOUND BY ENZYMES THAT COVALENTLY MODIFY SUBSTRATE MOLECULES

(57) Abstract

This invention provides for the active site mapping of enzymes which catalyse covalent modification including, but not limited to phosphorylation, acylation, dephosphorylation in which a fixed residue (known as the catalytic residue) such as a tyrosine, serine, threonine, histidine, aspartic acid residue or any other residue containing an appropriate side chain is modified. Mapping of protein kinases is exemplified. The method of the invention has an additional level of complexity over and above that of the self-deconvoluting libraries described in WO97/42216. This involves making a library of smaller libraries (referred to as subsets) where a fixed residue is moved stepwise through the sequence of amino acids or other groups (such as peptidomimetics). Using 5 subsets of libraries of peptides of 5 amino acids allows the mapping of a sequence of 9 amino acids. In general one could carry out the invention using n subsets of n-mer peptides so as to provide mapping data for the residues from -(n-1) to +(n-1) either side of the active site. Thus in general the length of the mapped sequence would be (2n)-1. In this invention there is no need to separate modified from unmodified sequences because of the self deconvoluting nature of the library. The assay screen produces a series of hits, the patterns of which reveal the unique sequences in each well. This enables a pattern of substrate preferences to be determined for any enzyme. The unique sequences obtained using this invention can be used to provide substrates for high throughput assays and provide detailed information about the active site to aid rational drug design. This invention can also be used as an inhibitor library to screen against known modifying enzymes where a known substrate exists and can be set up in an assay format.